Vaccine 32S (2014) A10–A12

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Multi-center surveillance of rotavirus diarrhea in hospitalized children <5 years of age in India, 2009–2012

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A B S T R A C T

Diarrheal disease due to Group A rotaviruses continues to be an important cause of morbidity in the developing world and India contributes significantly to the disease burden. Surveillance carried out between July 2009 and June 2012 at two medical centers in south India and one center in north India estimated 39% of all diarrheal admissions to be due to rotavirus. The most prevalent genotype isolated was G1P[8](33%) followed by G2P[4](17%). G9P[4] has also emerged as a significant cause of rotavirus diarrhea. No seasonal variation was noticed from the centers in south India, whereas we observed increased rotavirus diarrhea in the center in north India during March and April.

1. Introduction

Group A rotavirus remains one of the leading etiological agents of infectious diarrhea in children <5 years of age, in developing countries. India contributes to 22% of rotavirus diarrhea related mortality in the world [1]. A previous multi-center study under the Indian Council of Medical Research (ICMR) and US Centers for Disease Control and Prevention (CDC) showed that 40% of the diarrheal admissions were attributable to rotavirus [2,3].

Two vaccines against rotavirus based on immunogenicity testing, Rotarix and Rotateq, are licensed and available in India [4,5]. While phase II/III trials for other candidate vaccines are ongoing [6], it is important to monitor the burden of rotavirus diarrhea in India to gauge the effectiveness and impact of vaccines, when and where they are used, and possibly to monitor the emergence of strains under vaccine pressure.

We conducted a multicenter hospital-based surveillance from July 2009 to June 2012 to determine the burden and molecular epidemiology of diarrheal disease due to rotavirus.

2. Material and methods

The Christian Medical College (CMC), Vellore, Child Jesus Hospital (IJH), Trichy, and St. Stephen's Hospital (SSH), Delhi took part in hospital-based surveillance from July 2009 to June 2012 at CMC and IJH and July 2009 to June 2011 at SSH, following the previously described protocol [2]. Briefly, all children <5 years of age, admitted with a diagnosis of diarrhea were approached for participation in this study. After obtaining informed consent, a stool sample was collected within 24 h of admission. Stool samples were shipped to CMC at 4 °C every 15 days. The study was approved by the institutional review board (IRB) of the participating centers.

2.1. Sample testing

All the stool samples were shipped to the testing laboratory (CMC) at 4 °C. Samples were tested for the presence of rotavirus using a commercially available antigen detection ELISA (Premier™ Rotaclone®, Meridian Biosciences) as per kit protocol. Samples showing an OD value of ≥0.150 were reported as positive. An internal control was included in all runs, and the run was repeated if the internal control did not fall in the expected range.

2.2. Genotyping samples

Genotyping was performed on the antigen positive samples. RNA was extracted using the QIAamp Viral RNA Mini Kit. Complementary DNA was synthesized using random primers (Pd(N)6 hexamers; Pharmacia Biotech) and 400 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies) and was used as template for VP7 and VP4 (G and P) typing in PCRs using published oligonucleotide primers and protocols to

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http://dx.doi.org/10.1016/j.vaccine.2014.03.001

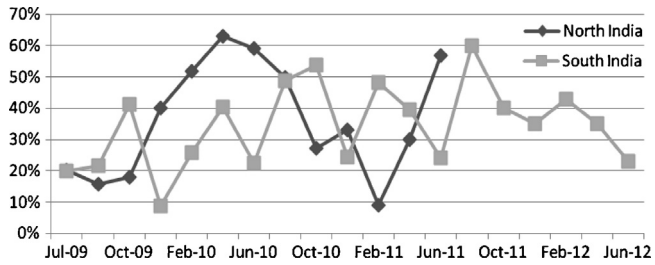

Fig. 1. Seasonal trend of rotavirus positivity at two sites in south India and one site in north India. The sites in south India participated from July 2009 to June 2012. The north Indian site participated from July 2009 to June 2011.

Table 1
Genotypes isolated during surveillance of rotavirus gastroenteritis in children less than 5 years of age hospitalized in India between July 2009 and June 2012.

	P4	P6	P8	Other P	P untyped	Mixed P	Total
G1	2	4	133	0	18	10	168
G2	69	0	0	0	3	1	73
G9	43	0	25	0	4	1	73
G12	0	25	14	0	2	0	41
Other G	0	0	0	0	1	0	1
G untyped	1	1	5	0	9	0	15
G mixed	9	4	2	0	5	4	24
Total	124	34	179	0	42	16	395

detect VP7 genotypes G1, G2, G3, G4, G8, G9, G10, and G12 and VP4 genotypes P[4], P[6], P[8], P[9], P[10], and P[11] [2].

Samples which failed to type the first time were confirmed to be rotavirus positive by PCR to detect the VP6 gene. If the VP6 PCR was positive, alternate primer sets were used to attempt genotyping. Samples which were VP6 negative were re-extracted by Trizol method and subjected to a repeat VP6 PCR to confirm or rule out the presence of rotavirus [7].

3. Results

A total of 1191 children were recruited from the 3 sites over the study period and rotavirus was detected in 458 children using the antigen detection ELISA, accounting for 39% of the cases of diarrhea. The detection rates of rotavirus varied from 26% in Vellore to 40% in Delhi and 50% in Trichy. The proportion positive each year did not vary by site, with higher rates in Trichy and lower rates in Vellore in each year of surveillance. Of the children recruited, 60% were male, with mean age of 10.1 months (±SD 7.4) versus 40% female with an average age of 11.6 months (±SD 7.6). The median age of rotavirus positive and negative cases was 10 months. Of the children who tested positive for rotavirus, 63% were less than 1 year of age, 26% 1–2 years of age and 11% between ages of 2 and 5 years.

Rotavirus was detected throughout the year from the sites in south India compared to the site in the north India where the rates of detection where much higher during March–April, as compared to the other months (Fig. 1).

Of the 458 samples which tested positive by ELISA, genotyping was attempted for 453 strains (98%). Fifty-eight (13%) of the ELISA positive samples were negative on genotyping, and when tested for VP6 gene they were all negative even after re-extraction of samples by another method (Fig. 2a). Of the 395 samples, 96% were G-typed and 91% were P-typed. Both G and P type was obtained for 315 (80%) strains. The most prevalent G and P type combinations were G1P[8] (133/395, 33%), G2P[4] (69/395, 17%) and G9 P[4] (43/395, 11%) (Fig. 2b, Table 1). We detected G12 strains, in combination with P[6] and P[8], from both the north and south Indian sites, with more G12 P[6] strain from north India. Mixed infections were seen

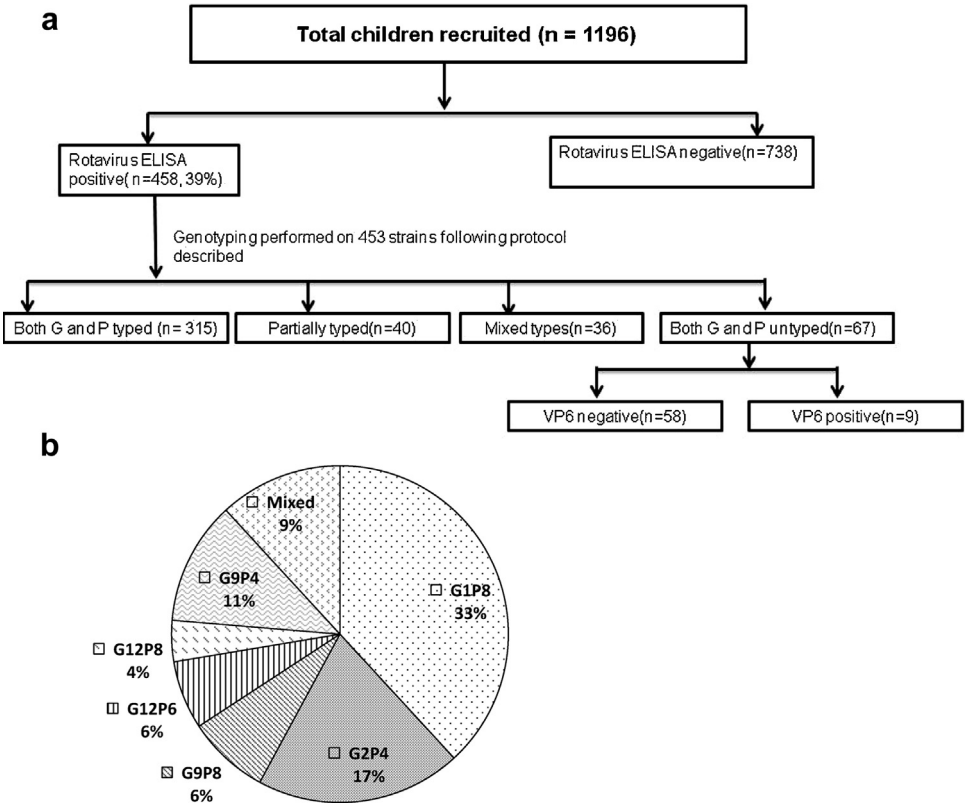

Fig. 2. (a) Samples tested and results obtained from the three sites in India between July 2009 and June 2012 from children hospitalized with acute gastroenteritis. (b) Genotype distribution of rotaviruses identified at three sites in India between July 2009 and June 2012 from children hospitalized with acute gastroenteritis.

in 9% (n = 36) of the strains which could be typed, G1 in combination with either G9 or G12 were the most common.

4. Discussion

This study demonstrates the high prevalence of rotavirus diarrheal disease related hospitalizations in India. The rates are comparable to other hospital-based studies across India which have demonstrated a similar burden of disease. A recent review estimated that rotavirus hospitalizations ranged from 19.2% in Lucknow to 49.9% in Manipur [8]. The results from the previous network surveillance conducted from 2005 to 2009 across various hospital sites in India, showed rotavirus positivity rates ranging from 35% in western India to 44% in south India [2,3].

The study showed a 39% isolation of rotavirus both from south and north India. In Trichy, 50% of samples tested were positive for rotavirus. There was no definite seasonal pattern in south India, where sites have had a stable proportion of rotavirus over 3 years. In northern India, the rates of detection were higher in the months of March–April for 2 years of surveillance. This differs from previous studies, which showed an earlier peak in rotavirus diarrhea in December to February in north India [2,3,9].

G1P[8] was the most commonly identified genotype, which follows the trend seen during the previous surveillance conducted from 2005 to 2009 [2,3]. The continued isolation of G12 strains shows the establishment of these strains in the Indian population. G9P[4] was the third most common strain to be isolated. This is in contrast to the previous report, where the isolation of G9P[4] was occasionally reported and the P[8] strain was the predominant associated P type for G9 strains [2,3]. Other sites within India have also reported the increased isolation of G9P[4] strains from their regions [10,11].

The false positivity rates (13%) obtained by the antigen detection ELISA were high. This is a cause for concern because in prior studies, rates of false positivity with diarrheal samples have been less than 10%. To differentiate the truly untyped samples from the negative samples, we repeated extraction and performed PCR to detect the VP6 gene, by two different methods, and the samples remained negative. The majority of the samples with negative PCR result were borderline positive by ELISA. One explanation is the possible degradation of the nucleic acid during transport. Our results indicate the need for close monitoring of ELISA results – commercially available antigen detection ELISAs being the common method for rotavirus detection – and inclusion of additional internal controls.

Surveillance to document the rates of rotavirus related diarrhea and the strain distribution is important. The World Health Organization recommends the use of rotavirus vaccines to prevent severe rotavirus gastroenteritis globally [12]. Although vaccine efficacy is lower in developing countries, the effectiveness of the vaccines in decreasing the large public health burden of acute gastroenteritis supports their use [13]. The indigenous development of vaccines is expected to decrease the price and increase the uptake, particularly if vaccines can be included for routine childhood immunization. The increase in availability and use of rotavirus vaccines in the future underlines the importance of surveillance networks to investigate the post-vaccine introduction epidemiology of rotavirus in terms of disease burden and effect on strain types.

Funding

Sudhir Babji was supported by the Global Infectious Disease Research Training Grant (D43TW007392; PI - GK).

Conflict of interest statement

None of the authors report a conflict of interest.

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